Studies of Myosin in Hereditary Muscular Dystrophy in Mice *

MARVIN SMOLLER † AND RICHARD A. FINEBERG ‡

(From the Department of Biochemistry, University of California Medical Center, San Francisco, Calif.)

In the light of current genetic knowledge, investigation of the primary abnormality of a hereditary disease is logically directed toward a genetically abnormal protein. Biochemical studies of hereditary human and animal progressive muscular dystrophy have revealed a variety of abnormalities such as elevated serum enzymes and lowered muscle enzyme activity, increased creatine excretion, and lowered muscle potassium content, which have been recently reviewed (1, 2). These changes are, however, generally considered to be secondary to muscle destruction (2-4). A muscle protein abnormality has been suggested in recent studies by Kruh, Dreyfus, Schapira, and Gey (5), and by Simon, Gross, and Lessell (6), showing an increased rate of muscle protein catabolism in hereditary dystrophy of mice. Destruction of the myofibrils, composed primarily of myosin and actin, has been shown to be an early event in dystrophy in the electron microscopic studies of Ross, Pappas, and Harman (7), and O'Steen (8, 9) has demonstrated in tissue culture that myofibrils from dystrophic mice regenerate abnormally. In earlier investigations on isolated myosin, Schapira, Joly, and Dreyfus (10) showed by light scattering that the protein from dystrophic human muscle had more variable particle size, and Horvath (11) in an abstract reported greater polydispersity on the basis of the ultracentrifuge pattern of myosin from human dystrophic muscle.

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Aided by a grant from the Academic Senate of the University of California Medical Center.

† Recipient of a fellowship from the Muscular Dystrophy Association of America. Present address: Department of Medicine, University of California, San Francisco General Hospital.

‡ Address requests for reprints to: Dr. Richard A. Fineberg, Dept. of Biochemistry, University of California School of Medicine, San Francisco, Calif.

In view of these previous studies, and the fact that myosin is the major protein of muscle, the purpose of the present work was to determine whether this protein differed from normal in hereditary muscular dystrophy. Mice were selected as a source of myosin because of the constant availability of these animals in early stages of the disease. Myosin was purified from dystrophic and normal mice of the same strain by the method previously described (12). The two preparations were compared in respect to physical and enzymic properties as well as certain aspects of the primary structure including total amino acid analysis and selective peptide fingerprinting. Marked differences were observed between myosin preparations from dystrophic and normal mice.

Methods

Purification of myosin. Homozygous normal and homozygous dystrophic mice of strain 129 were purchased.¹ Ten normal and 12 to 15 dystrophic mice yielding 60 to 90 g of ground carcass were used for each preparation. Animals were 8 to 9 weeks old when sacrificed. The mice were decapitated, skinned, eviscerated, chilled, and put through a meat grinder. The myosin was extracted for 10 minutes with 0.3 M KCl, 0.05 M histidine-HCl (pH 6.8) with 2 mM ATP, precipitated by diluting to 0.04 M KCl, collected by centrifugation, and redissolved in 0.5 M KCl, 1 mM Tris-HCl (pH 7.0). This solution is called stage one (Table I). Actomyosin was separated by centrifugation for 1 hour at $30,000 \times g$ after diluting the solution to 0.3 M KCl, and the myosin was then precipitated in 0.04 M KCl and dissolved in 0.5 M KCl, 1 mM Tris-HCl, pH 7.0 (stage 2, Table I). This solution was centrifuged (Spinco model L) for 2 hours at $100,000 \times g$, and the resulting clear solution was called stage 3 (Table I). The final purification step involved gel filtration on Sephadex G-200 to separate the large myosin molecules from contaminants smaller than approximately 200,000 mol wt. The myosin, identified by high ATPase specific activity and high A_{270}/A_{280} absorbancy ratio, was recovered in the void volume of the

¹ Jackson Memorial Laboratory, Bar Harbor, Maine.

TABLE I
Summary of the purification of myosin from normal and dystrophic mice*

Stage	Normal				Dystrophic			
	ATPase specific activity	Ultraviolet absorbancy ratio	ATPase total activity	Total protein	ATPase specific activity	Ultraviolet absorbancy ratio	ATPase total activity	Total protein
	<i>U</i> †	<i>A</i> ₂₇₉ / <i>A</i> ₂₈₀	<i>U/g</i> ‡	<i>mg/g</i> ‡	<i>U</i> †	<i>A</i> ₂₇₉ / <i>A</i> ₂₈₀	<i>U/g</i> ‡	<i>mg/g</i> ‡
1	30	0.823	830	27.7	19	0.710	645	34
2	40	0.832	236	5.9	30	0.812	300	10.1
3	59	0.944	231	3.9	40	0.877	252	6.3
4§	94 ± 7.7	1.28 ± 0.06	171 ± 23	1.9 ± 0.5	80 ± 7.8	1.23 ± 0.04	184 ± 15	2.3 ± 0.2

* Each value is the mean of five preparations; in the case of stage 4, the standard errors are shown. The stages of purification are described in the text.

† Micrograms P per milligram protein per 5 minutes.

‡ Yields expressed per gram of minced carcass.

§ None of the differences between the dystrophic and normal values are statistically significant.

column, and the two or three fractions with the highest enzyme activity and absorbancy ratios were pooled. This final solution, called stage 4 (Table I), was used in all further studies. Further details of this procedure for the extraction and purification of myosin (myosin A), including a comparison with other methods, appear in a previous communication (12).

Protein was determined spectrophotometrically assuming $A_{279}^{1\%} = 5.6$ as suggested for rabbit myosin by Small, Harrington, and Kielley (13). The absorbancy at 320 mμ was subtracted to eliminate nonspecific light-scattering effects. ATPase was measured by incubating a 0.1% solution of myosin for 5 minutes at 22° C in 0.5 M KCl, 0.06 M Tris-HCl (pH 9.0), 5 mM CaCl₂, and 5 mM ATP. Inorganic phosphate was determined after stopping the reaction with trichloroacetic acid (12).

Physical measurements. Sedimentation velocity studies were carried out in a Spinco model E analytic ultracentrifuge at 20° C and 50,740 rpm using a standard 4° cell and schlieren optics. Sedimentation coefficients were calculated as outlined by Schachman (14).

The viscosity of the stage 4 solutions was measured at 0° C in an Ostwald viscometer with a flow time of 146 seconds for 0.5 M KCl.

Fingerprinting of C¹⁴-labeled, SH-containing tryptic peptides. Four myosin preparations² from dystrophic mice and three from normal mice were reacted with *N*-ethylmaleimide-C¹⁴ (C¹⁴-NEM)³ and fingerprinted essentially as described by Kielley and Barnett (15). This method detects only the tryptic peptides that contain free SH and was chosen instead of the usual technique of Ingram (16) because the peptides expected from the tryptic hydrolysis of myosin are too numerous (*ca.* 256) for adequate separation.

Forty mg of 48-hour-old myosin in 0.5 M KCl, 1 mM Tris-HCl (pH 7.0) was made 2.0 M in guanidine-HCl by

adding the solid salt. To this solution (about 10 ml) was added 1.0 ml of C¹⁴-NEM (0.75 μc per μmole) containing 10 μc. The resulting solution was found to have a pH of 5.6, which is less favorable than pH 7 for the reaction (15). After 30 minutes at room temperature the resulting C¹⁴-NEM-S-myosin was precipitated by a 20-fold dilution with water. The precipitate was collected, washed 5 times with water, and placed in an automatic recording pH-stat⁴ with an N₂ barrier. After equilibration at pH 9.0, the protein was digested with 1.0 mg of trypsin added in 3 fractions over a 24-hour period. The proteolytic reaction was followed by the record of NaOH added to maintain the pH. On the average, 80% of the lysine and arginine peptide bonds (as calculated from the amino acid analysis) were split.

After concentration by drying *in vacuo* and redissolving, 1 to 2 mg of the tryptic peptides was subjected to electrophoresis on Whatman 3 filter paper 50 cm long in 0.05 M (NH₄)₂CO₃-NH₃ (pH 8.5) containing 1 mM EDTA for 75 minutes at 2,000 v (40 v per cm) in the apparatus described by Katz, Dreyer, and Anfinsen (17). After drying, the paper was chromatographed in butanol-pyridine-1 mM EDTA (3:3:4) in a second dimension for 7 to 8 hours. Radioautographs were made on X-ray film⁵ over 3 to 8 weeks.

Amino acid analysis. Myosin was dialyzed against water for 3 days, dried *in vacuo*, and oxidized by performic acid at 0° C according to the method of Hirs (18). From 2 to 5 mg of lyophilized, oxidized protein of each of three normal and three dystrophic preparations was hydrolyzed with 2 to 5 ml of redistilled 6.0 N HCl in Pyrex tubes evacuated by vacuum pump and sealed. Samples were removed after 20 and 70 hours of heating at 105° C. They were analyzed according to the Moore-Stein method as modified by Piez and Morris (19) on a Technicon amino acid autoanalyzer. The amount of protein applied to the ion-exchange column was determined by the micro-Kjeldahl method. Tryptophan was deter-

² The preparations will hereafter be frequently referred to as normal and dystrophic myosin for simplicity of expression, but with no intent to anticipate the conclusion.

³ Schwartz Biochemicals, Orangeburg, N. Y.

⁴ Radiometer, Type TTT 1c, Copenhagen, Denmark.

⁵ Kodak Blue Brand, Eastman Kodak Co., Rochester, N. Y.

TABLE II
Sedimentation and viscosity of myosin preparations
from normal and dystrophic mice (mean* $\pm$ SD)

Animals	Sedimentation coefficient		Intrinsic viscosity [η]
	Slow component	Fast component	
	$S_{20,w}$		dl/g
Normal	6.75 $\pm$ 0.05	7.4 $\pm$ 0.6	1.9 $\pm$ 0.1
Dystrophic	6.79 $\pm$ 0.05	52 $\pm$ 0.7	2.6 $\pm$ 0.2

* Each mean represents three preparations in the case of the ultracentrifuge data and four in the case of viscosity.

mined spectrophotometrically by the method of Beaven and Holiday (20).

Determination of free sulfhydryl groups of the native protein was performed by the method of Boyer (21) using *p*-chloromercuribenzoate (PCMB). A 24-hour reaction time was used to minimize the effects of variable reaction rates as suggested by Gilmour and Gellert (22). The temperature of incubation was 4° C. No correction was made for light-scattering due to aggregation. A second estimate of free sulfhydryl was made by comparing the binding of C^{14} -NEM per mg of protein after the 30-minute reaction as described in the section on fingerprinting. C^{14} was determined in a scintillation counter.⁶

Results

Purification. Contrary to previous reports (23–25) the average amount of myosin isolated per gram of minced dystrophic mouse carcass was similar to that isolated from normal mice (Table I). The better yield may be due to the minimal muscle destruction in the 7- to 8-week-old animals used, and perhaps to the use of ATP in the extraction medium. The final degree of purification, as measured by specific activity of ATPase and by absorbency ratio, was fairly comparable for the dystrophic and normal myosin, even though the extracts from dystrophic mice contained more contaminating material in the early stages of the isolation.

Myosin from each type of animal retained 90 to 95% of its ATPase activity after 10 days of storage at 4° C. Thus the purified proteins did not show the more rapid decline in ATPase activity noted in dystrophic myofibril preparations by Kaldor and Gitlin (26).

Presumably because of the higher viscosity of the dystrophic myosin solutions (Table II), their

concentration in the gel filtration step had to be limited to about 0.6% to maintain a satisfactory flow rate. Another difference noted during the isolation procedure was that the initial dystrophic precipitate failed to flocculate as well as the normal.

Ultracentrifuge studies. Fresh preparations of dystrophic and normal myosin each showed a symmetric peak with a Svedberg sedimentation constant of 6.8 ($S_{20,w}$). However, the concentration dependence was somewhat less pronounced in the case of the dystrophic myosin (Figure 1); the negative slope of the least squares regression line of sedimentation coefficient vs. concentration was 0.4 times that of the normal. The two types of preparation differed further in that the normal contained an additional very small 7–8 S peak (< 1% of the total protein) (Figure 2A), whereas the dystrophic had a 52 S peak (Figure 3A and E) representing 20 to 30% of the total protein (Table II). These findings were consistent in all normal and all dystrophic preparations examined. The 7–8 S peak never appeared

FIG. 1. CONCENTRATION DEPENDENCE OF THE SEDIMENTATION COEFFICIENTS OF DYSTROPHIC ($\blacktriangle$) AND NORMAL ($\bullet$) MOUSE MYOSIN. The two lines at the bottom represent the main ultracentrifugal peaks, and the upper two are the faster sedimenting minor components. The arrows indicate the effect of ATP on two of the samples of dystrophic myosin.

⁶ Tri-Carb liquid scintillation spectrometer, Packard Instrument Co., La Grange, Ill.

FIG. 2. SEDIMENTATION PATTERNS OF NORMAL AND DYSTROPHIC MOUSE MYOSIN AND THE EFFECT OF COLD STORAGE. Sedimentation from right to left. Solvent: 0.5 M KCl, 1 mM Tris-HCl (pH 7.0). Speed: 50,740 rpm. Temperature: 20° C. A. 24-HOUR-OLD NORMAL MYOSIN. Concentration: 4.34 mg per ml. Time: 32 minutes. Bar angle: 60°. In spite of the high concentration, no more than a trace of 7.5 S material sedimenting ahead of the main peak is discernible in this fresh myosin preparation. B. 8-DAY OLD NORMAL MYOSIN. Concentration: 2.53 mg per ml. Time: 88 minutes. Bar angle: 60°. This aged preparation reveals an increase in the relative amount of the faster component. C. 10-DAY OLD DYSTROPHIC MYOSIN. Concentration: 2.86 mg per ml. Time: 3 minutes. Bar angle: 60°. This photograph taken soon after the rotor reached high speed shows the 52 S component that was present in addition to the 6.85 S component in all of the dystrophic myosin preparations. D. SAME SAMPLE AS C. Time: 56 minutes. Bar angle: 50°. The 52 S peak has reached the bottom of the cell, and a new peak (3-4 S) has appeared behind the main peak. The slow component was found only in preparations stored for 8 to 10 days.

in any dystrophic preparation nor the 52 S peak in a normal preparation.

There was also a difference in the effect of storage at 4° C. In the normal the slightly faster component (7-8 S) increased in amount with time (Table III and Figure 2B). This suggests a continuing aggregation as noted with myosin of other species, e.g., by Connell (27). The rapid 52 S component of the dystrophic myosin, however, remained at approximately the same percentage of the total protein (Figures 3A and 2C). Furthermore, in the dystrophic preparation only, a new component (3-4 S) was also seen after 10 days of storage (Figure 2D).

The myosin preparations from dystrophic mice differed also in their response to added ATP. Whereas the ultracentrifuge pattern of normal myosin was unaffected, the dystrophic preparations showed marked changes. The 52 S component decreased to 3% (Table IV and Figure 3), and the S value for this small remnant of fast-sedimenting material was decreased (Figure 1). The 6.8 S peak increased correspondingly from 71% to 96% of the total protein. Since there was no appearance of new peaks that could

represent actin, nor any significant decrease in the total protein in the two main peaks, it is unlikely that the ATP-induced change was due to an actin-myosin dissociation. Rather it is more likely due to a dissociation of aggregated myosin to the monomer. Gellert, von Hippel, Schachman, and Morales (28) have demonstrated that ATP dissociates "polymyosin" to monomeric myosin molecules.

Amino acid analysis. Results of total amino acid analysis of three dystrophic and three normal myosin preparations are shown in Table V. There were five amino acids in which the mean number of residues found in the myosin from dystrophic animals differed by a statistically highly significant amount ($p < 0.01$) from that of the myosin from the normal animals. These differences included increases in Ala [15],⁷ Val [8], and Ileu [8] and decreases in Gly [7] and His [4]. Phe also showed a decrease [4] although only at the 95% confidence limit. The percent difference from normal in the case of these six amino acids averaged 20% ($\pm 5\%$ SD).

⁷ Bracketed numbers indicate number of residues (per 10⁶ g) differing from normal.

The increase in Asp [3] was not statistically significant, nor were the differences in Thr [1] and Arg [1]. The remaining nine amino acids found in dystrophic myosin differed by less than one residue and in each case by $\leq 2.5\%$ of normal. The ammonia was also found increased in the dystrophic myosin, but the significance of the increase is unclear since ammonia was subject to the greatest error of determination.

FIG. 3. EFFECT OF ATP ON THE SEDIMENTATION PATTERNS OF DYSTROPHIC MYOSIN. Sedimentation from right to left. Myosin, 24 hours old. Solvent: 0.5 M KCl, 1 mM Tris-HCl (pH 7.0). Speed: 50,740 rpm. Temperature: 20° C. Bar angle: 60°. A AND B. DYSTROPHIC MYOSIN WITHOUT ATP. Concentration: 2.86 mg per ml. Time: A, 6 minutes; B, 46 minutes. C AND D. SAME SAMPLE AS IN A AND B BUT WITH 1 mM ATP. Concentration: 2.57 mg per ml. Time: C, 7 minutes; D, 60 minutes. Comparison of C with A shows that ATP has decreased the rapid (52 S) peak and increased the 6.8 S peak. D and B show the increase in the main peak more clearly. E. DYSTROPHIC MYOSIN WITHOUT ATP. Concentration: 4.5 mg per ml. Time: 10 minutes. F. SAME SAMPLE AS E BUT WITH 1 mM ATP. Concentration: 4.0 mg per ml. Time: 7 minutes. The decrease in the rapid (52 S) peak under the influence of ATP is shown by comparison of F with E.

Fingerprinting. Radioautograms of triplicate fingerprint preparations made from four dystrophic and three normal preparations showed 15 to 17 consistent spots with no consistent difference between the dystrophic and normal material. The presence of 15 to 17 spots is similar to the findings for rabbit myosin by Kielley and Barnett (15) and Gröschel-Stewart and Turba (29).

TABLE III

Effect of storage at 0° C on the relative concentration of components of normal and dystrophic mouse myosin in the analytic ultracentrifuge (areas of peaks as per cent of total)

	Age	6.7 S peak	7.4 S peak	52 S peak	3-4 S peak
	days	%	%	%	%
Normal	1	99	1	0	0
	2	94	6	0	0
	4	87	13	0	0
	8	80	20	0	0
	10*	87	13	0	0
Dystrophic	1	77	0	23	0
	2	84	0	16	0
	4	80	0	20	0
	10	60	0	21	19

* Many rapidly sedimenting peaks appeared in this analysis.

The number of spots observed in the fingerprints, and the 15 to 16 cysteine residues per 175,000 g found on total amino acid analysis, are consistent with the existence of 3 identical myosin subunits, based on a myosin molecular weight of 524,000 (30).

Analysis of free sulfhydryl groups showed that the total number of free SH groups reacting with the C¹⁴-NEM and with the PCMB was less in the dystrophic myosin than in the normal (Table VI) even though the fingerprint patterns of the C¹⁴-NEM-S-peptides of both were similar. The 24-hour PCMB reaction showed 14 moles of free SH per 175,000 g of normal myosin. This is in

TABLE IV

Effect of ATP on the relative concentration of components of dystrophic mouse myosin in the analytic ultracentrifuge (areas of peaks as per cent of total)

Experiment no.	ATP	6.7 S peak	52 S peak	Change in total area of both peaks
		%	%	%*
1†	—	70	30	
	+	97	3‡	-3.7
2§	—	72	28	
	+	95	5‡	0.0

* Areas of samples with and without ATP were corrected to the same concentration. Areas were determined by cutting out and weighing enlarged tracings of the schlieren curves.

† Shown in Figure 3: without ATP (A and B); with ATP (C and D).

‡ After addition of ATP these peaks had an S value of 20 to 30.

§ Shown in Figure 3: without ATP (E); with ATP (F).

TABLE V
Total amino acid analysis of myosin from normal and dystrophic mice*

Amino acid	Content		Difference		p†
	Normal	Dystrophic	moles/10 ⁵ g recovered	%	
Cysteine‡	8.84 ± 0.15	8.90 ± 0.14	+0.06	0.7	
Methionine‡	26.8 ± 1.13	26.8 ± 1.21	0.0	0.0	
Aspartic acid§	82.5 ± 1.13	85.6 ± 1.06	+3.1	3.8	
Threonine	43.0 ± 0.05	44.4 ± 0.72	+1.4	3.3	
Serine	44.0 ± 0.70	44.4 ± 1.02	+0.4	0.9	
Glutamic acid	166.3 ± 0.52	166.5 ± 0.48	+0.2	0.1	
Proline	27.7 ± 0.92	27.6 ± 0.77	-0.1	0.4	
Glycine	53.0 ± 0.90	45.9 ± 0.81	-7.1	13.4	< 0.01
Alanine	63.9 ± 1.01	79.1 ± 0.66	+15.2	23.8	< 0.001
Valine¶	33.6 ± 0.65	41.7 ± 1.53	+8.1	24.2	< 0.01
Isoleucine¶	33.6 ± 0.40	41.4 ± 1.58	+7.8	23.3	< 0.01
Leucine	85.6 ± 0.70	85.1 ± 0.85	-0.5	0.6	
Tyrosine	19.1 ± 0.12	18.8 ± 0.28	-0.3	1.6	
Phenylalanine	33.4 ± 1.0	29.2 ± 1.21	-4.2	12.6	= 0.05
Lysine	81.8 ± 0.71	81.8 ± 1.24	0.0	0.0	
Histidine	19.4 ± 0.62	15.5 ± 0.32	-4.1	21.2	≈ 0.001
Arginine	43.2 ± 0.69	42.5 ± 0.91	-0.7	1.6	
Tryptophan**	8.1 ± 0.43	8.3 ± 1.1	+0.2	2.5	
Ammonia††	(39.0) ± 3.1	(79.1) ± 4.2			
Residues/10 ⁵ g recovered	873.8	893.5			
Weight/100 g‡‡	99.9 g	101.5 g			

* All values except those indicated are the means of four analyses, including 20-hour hydrolysates of three independent myosin preparations and a 70-hour hydrolysate of one of the preparations.

† Probability listed only for the significant differences.

‡ Cysteine was determined as cysteic acid and methionine as methionine sulfone, the protein having been oxidized with performic acid. The cysteine results were corrected for 10% loss and the methionine taken as 100% recovery as determined by Hirs (18).

§ Methionine and aspartic acid values are averages of only two analyses because of the poor separation of these two amino acids in the other analyses.

|| Threonine and serine were corrected for losses during hydrolysis by extrapolation of the 70-hour and the mean of the three 20-hour hydrolyses to zero time.

¶ Valine and isoleucine were determined from the 70-hour hydrolysis. Statistics were calculated from means of three 20-hour hydrolyses. The increase in each group from 20 to 70 hours was 20%.

** Tryptophan was determined independently on separate samples of four normal and four dystrophic preparations by the spectrophotometric method of Beaven and Holiday (20).

†† Ammonia was determined by averaging the three 20-hour hydrolyses and extrapolating the mean and the result of the 70-hour hydrolysis to zero time. Statistics were calculated from the means of the three 20-hour hydrolyses.

‡‡ Total calculated weight of anhydrous amino acid residues (exclusive of ammonia) recovered per 100 g protein applied to column. The data were corrected for the substitution of OH for NH₂ in the hydrolysis of the amide groups.

reasonable agreement with the number of spots found on the radioautograms and indicates that the 15 to 16 cysteic acid residues per 175,000 g

detected on amino acid analysis were probably all originally cysteine and not cystine groups.

Discussion

The data show consistent differences in the physical properties and amino acid composition of highly purified myosin preparations from hereditary dystrophic mice as compared with those from normal mice of the same strain.

The ultracentrifuge studies suggested that 20 to 30% of the dystrophic myosin was present as large aggregates (52 S) under conditions where normal myosin was present almost entirely as monomers (6.8 S). The aggregates of dystrophic myosin were apparently broken down to monomers by ATP. The presence of the large aggregates accounts for the increased intrinsic vis-

TABLE VI

Determination of free sulfhydryl groups of normal and dystrophic mouse myosin by reaction with *p*-chloromercuribenzoate and *N*-ethylmaleimide-C¹⁴

	Dystrophic	Normal	Ratio
	moles SH/175,000 g myosin		
By PCMB (24 hr)*	9.6	13.9	0.60
By NEM-C ¹⁴ (30 min)†	5.25	7.75	0.60

* *p*-Chloromercuribenzoate reaction measured by ΔOD at 255 mμ after 24-hour incubation, pH 7.0, 0.5 M KCl, at 4° C.

† *N*-ethylmaleimide-C¹⁴ reaction measured by specific radioactivity of the protein after incubation with excess reagent for 30 minutes in 2.0 M guanidine-HCl, 0.5 M KCl, pH ca. 5.6.

cosity of dystrophic myosin and its decreased mobility in gel filtration. Studies during 10 days of cold storage showed that the dystrophic myosin solutions retained the same ultracentrifuge pattern until 8 to 10 days when a slower-moving component appeared. Since 95% of the ATPase activity remained in these 10-day samples, the presumed degradation products retained their enzyme activity. In contrast, the myosin from normal mice under the same storage conditions slowly aggregated to 7.5 S material that was not affected by ATP; slower sedimenting components did not appear.

Another striking difference noted in myosin from dystrophic animals was that fewer SH groups reacted with sulfhydryl reagents than in myosin from normal mice. Since both myosins were found to have the same number of cysteine residues (as cysteic acid) on total amino acid analysis, a difference in content of SH-containing amino acids was excluded. One possible explanation for the difference in SH reactivity could be that S-S bonds were present between some of the cysteine groups. The close similarity of the C^{14} -NEM-S-peptide fingerprints would make the S-S bond explanation unlikely unless these bonds were completely random through the molecule. A second possibility is that conformational alteration of the dystrophic molecule made some of the SH groups unavailable for reaction. Either mechanism might account for the high percentage of aggregates found in the dystrophic myosin.

The most basic difference between the myosin preparations from dystrophic and normal mice was in the total amino acid analysis. The observed differences from normal both in the aggregation and in the amino acid composition of the dystrophic myosin preparations could conceivably be due to differences in purity, although the preparations met the criteria of purity applied. They were similar in absorbancy ratio (A_{279}/A_{260}) and ATPase specific activity, and the ultracentrifuge patterns appeared to contain no nonmyosin peaks, since the fast component in the dystrophic preparations apparently represented myosin aggregates almost completely dissociable by ATP. These criteria, of course, are not sufficient to exclude the presence of some impurities. If the degree of purity is sufficient, then the striking difference in amino acid com-

position would indicate a difference in myosin structure. This may result from a mutation in the genes for this protein, even though the number of significant differences would imply the operation of a different genetic mechanism from the point mutation of a structural gene observed in many hemoglobinopathies (31).

The observations are therefore consistent with the proposition that progressive muscular dystrophy of the mouse may be due to a gene mutation producing myosin with altered structure. The structural alteration could involve surface or conformational changes in the molecule leading to the altered *in vitro* properties of dystrophic myosin and might in the live muscle result in more rapid breakdown of the myosin than normal. Although muscular dystrophy of the mouse may not be completely analogous to human progressive muscular dystrophy, a similar mechanism could also be operative in the human disease.

Summary

Myosin was isolated from mice with hereditary muscular dystrophy (strain 129, Jackson Memorial Laboratory) and from normal mice of the same strain. The following differences were observed: 1) the myosin from dystrophic animals was found to have a higher content of alanine, valine, and isoleucine and a lower content of glycine and histidine; 2) about 25% of the protein appeared to be large aggregates under conditions where the normal was almost entirely monomeric; 3) fewer of its SH groups reacted with sulfhydryl reagents than normal despite similar total cysteine content and similar number of SH-containing tryptic peptides; 4) it seemed less stable than normal on storage.

It is suggested that the primary defect in muscular dystrophy of mice might be a genetic abnormality of the myosin molecule.

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